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Chemoprevention of prostate cancer by naturally occurring and synthetic organoselenium compounds

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14. ABSTRACT

The lack of treatment for "worried well" patients with high-grade prostatic intraepithelial neoplasia (HGPIN) combined with issues of recurrence and hormone resistance in prostate cancer survivors remains a major public health obstacle. Consequently, there is a strong need for mechanism-based naturally occurring or synthetic agents that can inhibit the development and/or progression of prostate cancer. Epidemiologic and preclinical studies, as well as some clinical intervention trials, show an inhibitory role of selenium against prostate cancer. The organoselenium compounds SM and *p*-XSC differentially interfere with prostate cancer cell growth and androgen receptor signaling (androgen receptor protein expression, phosphorylation, and PSA gene expression) in human prostate cancer cells. We have shown, for the first time, that inhibition of androgen receptor signaling by these compounds may be due, in part, to modulation of the mammalian target of rapamycin (mTOR) pathway. Our findings support the concept that dose and form are critical for the anti-cancer properties of selenium compounds and that these agents may be of value, either individually or in combination with other therapies, for the treatment of prostate cancer because of their potential to inhibit critical prostate signaling pathways.

15. SUBJECT TERMS

Selenium, prostate cancer, androgen receptor

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INTRODUCTION

Prostate cancer is the most commonly diagnosed malignancy and second leading cause of cancer-related death in men in the United States. The lack of treatment for "worried well" patients with high-grade prostatic intraepithelial neoplasia (HGPIN) combined with issues of recurrence and hormone resistance in prostate cancer survivors remains a major public health obstacle. Consequently, there is a strong need for mechanism-based naturally occurring or synthetic agents that can inhibit the development and/or progression of prostate cancer. Epidemiologic and preclinical studies, as well as some clinical intervention trials, show an inhibitory role of selenium against prostate cancer (1). The current study is investigating the mechanisms of inhibition of prostate cancer cell growth by the naturally occurring organoselenium compound selenomethionine (SM) and the synthetic selenium agent 1,4-phenylenebis(methylene)selenocyanate (*p*-XSC).

YEAR 1 PROGRESS REPORT

We proposed, in the Statement of Work for the first year of this award, to carry out studies described in Specific Aim 1 *[to elucidate the molecular mechanism by which the structurally distinct organolselenium compounds selenomethionine (SM) and 1,4-phenylenebis(methylene)selenocyanate (p-XSC) exert their anti-cancer effects on androgen responsive and androgen independent human prostate cancer cells]*, specifically sub-aims a through c (refer to Results section below). The following details the methodology employed as well as the progress made with respect to each of these sub-aims. In addition, we describe the results of preliminary findings pertaining to aim 1d, which was proposed to be addressed during the second year of the award.

MATERIALS AND METHODS

Reagents and cell lines

SM was purchased from Sigma Chemical Company (St. Louis, MO) and the synthetic organoselenium compound, *p*-XSC was synthesized as reported previously (2). Androgen responsive (AR) LNCaP cells were obtained from the American Type Culture Collection (Manassas, VA) and androgen non-responsive or androgen independent (AI) LNCaP C4-2 cells were obtained from Dr. Warren D.W. Heston, The Lerner Research Institute, The Cleveland Clinic Foundation, OH. LNCaP C4-2 cells were derived from cultures with bone stromal fibroblasts under androgen-deprived conditions and express comparable levels of androgen receptor to AR LNCaP cells but are unresponsive to androgen stimulation (3).

The AR LNCaP cells retain many differentiated features of normal human prostatic epithelial cells such as expression of estrogen receptor beta and a functional, although mutated, androgen receptor, the ability to secrete PSA and prostatic acid phosphatase (PAP) and expression of redox sensitive wild-type p53 tumor suppression gene and protein. Use of the androgen independent LNCaP C4-2 variant offers the ability to compare patterns of biomarkers and genomic responsiveness to organoselenium compounds to those obtained in the parent LNCaP cells.

Cell culture

AR cells were maintained in RPMI-1640 medium supplemented with non-essential amino acids, 2 mM glutamine, and 10% heat-inactivated Fetal Bovine Serum (FBS). AI cells were maintained under the same conditions but with 10% FBS. Cells were routinely passaged when they were 70-80% confluent. Cells were plated in 100 mm dishes containing 10 mL of medium and incubated at 37 ^{o}C in a humidified atmosphere of 5% CO_2. Following treatments, cells were rinsed with phosphate buffered saline (136.9 mM NaCl, 2.68 mM KCl, 8.10 mM Na_2HPO_4, 1.47 mM KH_2PO_4, pH 7.34, PBS) to remove debris and damaged cells. Only adherent prostate cells that maintained viability were harvested from plates by either trypsinization or gentle scraping into PBS and centrifuged at 500 x g to obtain a cell pellet and washed twice with 5 mL volumes of PBS.

Organoselenium treatments

Both AR and AI cells were incubated for varied amounts of time in media containing an organoselenium compound in a dose range of 0 – 100 μM. The vehicles for the naturally-occurring SM and the synthetic *p*-XSC were saline and dimethylsulfoxide (DMSO), respectively. After incubation, cells were harvested by trypsinization or scraping and cell counts and viability were determined.

Cell Death ELISA

AR and AI cells were plated in 96-well format (10,000 cells per well). Following treatment for 24 hr with 10, 50 and 100 μM SM or 2.5, 5, and 10 μM *p*-XSC, cells were assayed for the presence of cytoplasmic

histone-associated DNA fragments characteristic of apoptosis using the Roche Cell Death ELISA kit (Basel, Switzerland), according to the manufacturer's instructions.

Immunoblotting

AR and AI cells were treated with SM (5, 10, 50 and 100 μM) or *p*-XSC (5, and 10 μM) for 24 hours, harvested by scraping and washed with phosphate buffered saline. Protein extraction was carried out using cell lysis buffer (20 mM Tris pH 7.5, 150 mM NaCl, 1mM EDTA, 1 mM EGTA, 1% Triton, 2.5 mM Sodium pyrophosphate, 1 mM β-Glycerophosphate, 1 mM Na_3VO_4, 1 μg/ml Leuptin) with freshly added 1 mM PMSF. Equal amounts of protein (35 μg) were separated on 10% SDS-PAGE gels and transferred to nitrocellulose or PVDF membranes. Antibodies used for immunoblotting were Akt, phospho-Akt (Ser473), PI3K p85, cleaved PARP (Asp214), mTOR, phospho-mTOR (S2481), Raptor, Rictor, and androgen receptor from Cell Signaling Technology (Beverly, MA), phospho-androgen receptor (Ser210) from Imgenex Corp, (San Diego, CA) and GAPH from Santa Cruz (Santa Cruz, CA). Band expressions were developed using ECL reagents from Amersham (Piscataway, NJ) or Millipore (Billerica, MA).

Akt kinase activity assay

AR and AI cells were treated with SM (50 and 100 μM) and *p*-XSC (5 and 10 μM) for 6 hours, harvested by scraping, lysed, and assayed for Akt activity using the Cell Signaling Akt Kinase Assay according to the manufacturer's instructions. Briefly, Akt was immunoprecipitaed by incubating 200 μg protein lysate from each sample with 20 μl immobilized Akt antibody with rocking overnight. After washing, the *in vitro* kinase assay was performed by incubating the sample for 30 min at at 30 ^{o}C with ATP and a GSK-3 fusion protein as a substrate. The samples were then separated on a 12% SDS-PASE gel, transferred to PVDF membrane and probed for phospho-GSK03α/β (Ser21/9).

Immunoprecipitation

AR and AI cells were treated with SM (5 and 10 μM) and *p*-XSC (2.5, 5 and 10 μM) or rapamycin (1 nM) for 6 hours, harvested by scraping, and lysed. Protein lysate (200 μg) from each sample was incubated overnight with rocking with mTOR antibody (Cell Signaling, Beverly, MA) at a dilution of 1:100. Next, 20 μl of a Protein A agarose slurry (Invitrogen, Carlsbad, CA) was added and the samples were incubated with rocking for 3 hours. The samples were then pelleted, resuspended in SDS sample buffer and separated on 7.5% SDS-PAGE gels. The protein was transferred to PVDF membranes and analyzed by immunoblotting.

Transcription factor activity ELISA

AR and AI cells were treated with *p*-XSC (2.5 and 5 μM) for 24 hours, and nuclear extracts were prepared using the Cayman (Ann Arbor, MI) nuclear extraction kit according to the manufacturer's instructions. Recombinant Sp1 protein (Promega, Madison, WI) was incubated with 2.5 or 5 μM *p*-XSC for 30 minutes. Sp1 activity from nuclear extracts were measured using the TransAM™ transcription factor assay kit (ActiveMotif, Carlsbad, CA) according to the manufacturer's instructions. Briefly, 5 μg of nuclear or recombinant protein was added, in triplicate, to a 96-well assay plate and incubated for 1 hour with complete binding buffer. After washing, Sp1 antibody (1:1000) was added to the plate and incubated for 1 hour at room temperature. The plate was then washed and an HRP-conjugated anti-rabbit antibody was added. After incubation for an hour at room temperature, the plate was washed and incubated for 5 minutes with a developing solution followed by a stop solution. The absorbances at 450 nM were read and results expressed as percent A_{450} of control samples.

RESULTS

Specific Aim 1a: Determine the dose response of SM and p-XSC on induction of apoptosis in AR and AI prostate cancer cells

Summary*: p-XSC, but not SM, induces apoptosis in AR and AI prostate cancer cells*

Details: Apoptosis is a critical cellular event in cancer chemoprevention by selenium compounds (4). SM and *p*-XSC have been shown previously to inhibit LNCaP and C4-2 cell growth; however their effects on apoptosis have not been determined (5). We investigated the effect of SM and *p*-XSC on apoptosis in AR and AI cells using Cell Death ELISA. *p*-XSC induced 2.5-, 3.7-, and 5.8-fold increases in apoptosis in AR LNCaP at concentrations of 2.5, 5, and 10 μM, respectively (Figure 1A). Similarly in AI C4-2 cells, *p*-XSC induced 2.9-, 3.5-, and 4.4-fold increases in apoptosis at concentrations of 2.5, 5, and 10 μM, respectively (Figure 1B). SM showed no induction of apoptosis in either cell line at concentrations up to 100μM. PARP cleavage serves as a marker for apoptosis because it is one of the major targets of caspase-3 *in vivo* (6,7). Western blot analysis of

cell lysates from AR and AI cells shows increased levels of cleaved PARP in cells treated with 5 and 10 μM *p*-XSC (Figures 1A and 1B). AR and AI cells treated with doses of SM ranging from 5 to 100 μM show no detectable PARP cleavage, supporting the results from the Cell Death ELISA that SM lacked an effect on apoptosis in these cells. Taken together these results show that *p*-XSC significantly and dose-dependently induces apoptosis similarly in AR LNCaP and AI C4-2 cells and that inhibition of cell growth by *p*-XSC (5) is due, at least in part, to programmed cell death.

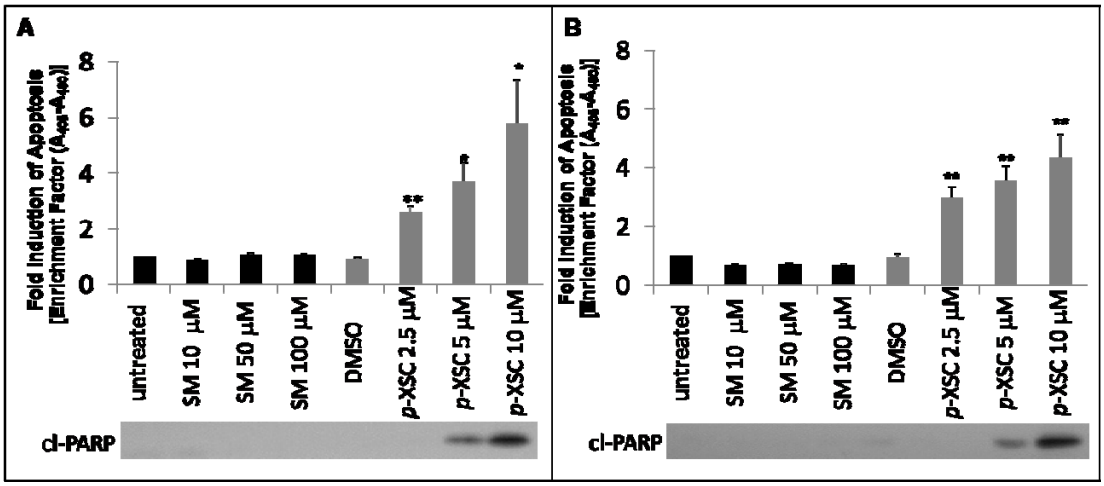


Figure 1. Apoptosis was measured by Cell Death ELISA and by cleaved PARP (cl-PARP) levels in A. AR and B. AI cells treated with SM and p-XSC. *p<0.05, **p<0.01

Specific Aim 1b: Measure, by immunoblotting, the effects of SM and p-XSC on the levels of androgen receptor and other proteins implicated in the regulation of androgen receptor signaling.

Summary*: p-XSC reduces androgen receptor levels in AR and AI cells, but the effects of SM are limited to AR cells.*

Details: Androgen receptor mutation, overexpression, and gene amplification contribute to prostate cancer progression and the development of androgen independent disease (8). Selenium compounds [e.g., methylseleninic acid (MSeA) and Se-methylselnocysteine (MSC)] have been shown to decrease androgen receptor mRNA and protein levels in a variety of prostate cancer cell lines and in an *in vivo* mouse model of prostate cancer (9-13). To determine the effect of SM and *p*-XSC on androgen receptor and androgen receptor signaling, we first examined the effects of these compounds on androgen receptor protein levels in AR and AI cells. SM and *p*-XSC significantly reduced androgen receptor protein levels in AR cells though *p*-XSC shows a greater reduction in expression (Figure 2A). *p*-XSC significantly reduces androgen receptor protein levels in AI prostate cancer cells, while SM shows no effect (Figure 2B).

Summary*: SM and p-XSC affect androgen receptor phosphorylation but not Akt signaling*

Details: Androgen receptor phosphorylation is thought to play a role in the regulation of its function. Phosphorylation of nuclear receptors can facilitate recruitment of co-regulators, modulate growth stimulatory effects, and target proteins for ubiquitination/degradation. Akt phosphorylates androgen receptor at specific serine residues (S210, S790) (14-16). PI3K/Akt signaling is upregulated in AI compared to AR prostate cancer cells and upregulation of this pathway correlates with prostate cancer progression and has been shown to promote androgen

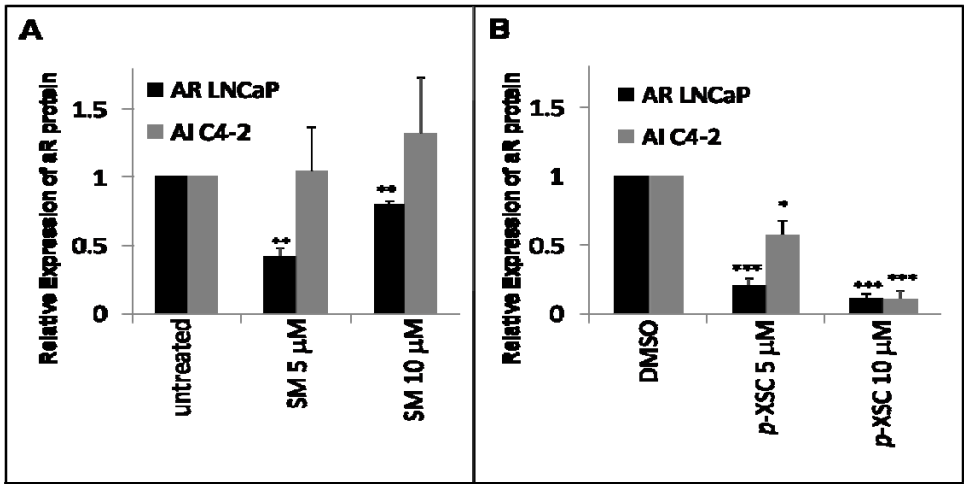


Figure 2. Androgen receptor levels in whole cell lysates from AR and AI cells treated with A. SM and B. *p*-XSC were measured by western blot, normalized to GAPDH protein and results expressed relative to control samples. *p<0.05, **p<0.025, ***p<0.005

escape (17-20). Various selenium compounds (e.g., MSeA, selenite) have been shown to target Akt in prostate cancer cells lines (21-24). We investigated, by western blot analysis, the effects of SM and *p*-XSC on Akt-mediated phosphorylation of androgen receptor at serine 210 in AR and AI cells. Both *p*-XSC and SM, at doses of 10 µM and higher, reduced the levels of androgen receptor phosphorylated at Ser210 in AR cells (Figure 3A). No inhibitory effect, however, was seen in AI cells (Figure 3B). Additionally, neither compound showed a significant effect on the expression of Akt or on the phosphorylation of Akt at Ser473 (Figures 4A and 4B). We further examined the effects of SM and *p*-XSC on Akt kinase activity using an *in vitro* kinase assay (Cell Signaling, Beverly, MA). Again, neither SM nor *p*-XSC showed any direct inhibitory effects on the kinase activity of Akt (Figures 4C and 4D).

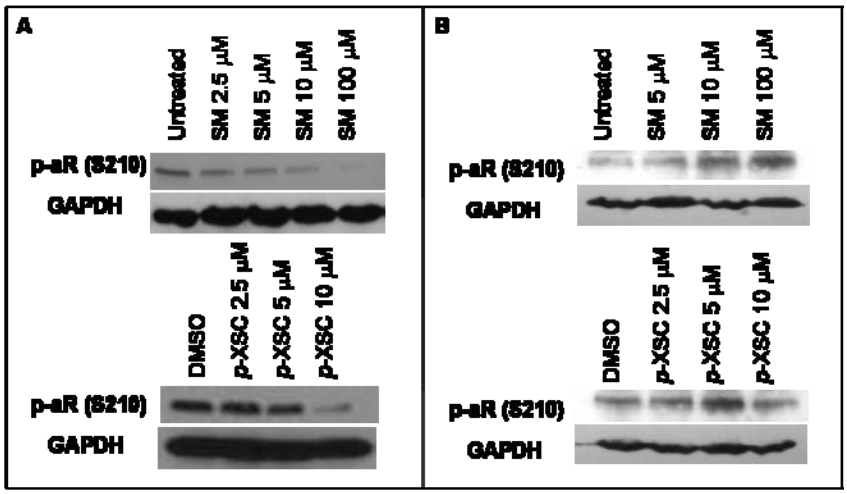


Figure 3. Western blot analysis of phosphorylated androgen receptor (p-aR) in whole cell lysates from A. AR and B. AI cells treated with SM and *p*-XSC.

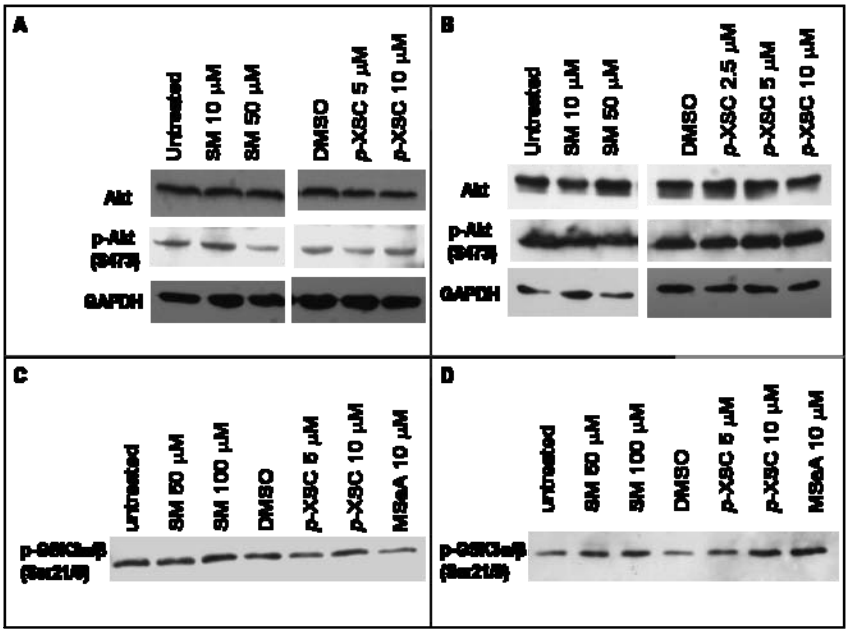


Figure 4. Western blot analysis of phosphorylated Akt (S473) in whole cell lysates from A. AR and B. AI cells treated with SM and *p*-XSC. *In vitro* Akt activity in C. AR and D. AI cell lysates was determined by measuring GSK3α/β phosphorylation.

***Summary**: SM and p-XSC modulate mTOR signaling*

Details: Along with the PI3K/Akt pathway, the mammalian target of rapamycin (mTOR) signaling pathway has been implicated in the progression of prostate cancer. Preclinical investigations point to mTOR signaling as an important regulator of prostate cancer cell growth and have linked the pathway to androgen receptor activity (25-27). To determine whether the mTOR pathway may be playing a role in the selenium-mediated down-regulation of androgen receptor, mTOR protein was immunoprecipitated from AR and AI cells that were treated with 5 and 10 µM doses of SM or *p*-XSC and analyzed for phosphorylation and binding to its protein complex partners Raptor and Rictor. Our results showed that *p*-XSC inhibits mTOR phosphorylation (Ser2481) in both AR and AI cells whereas SM showed decreased levels of phospho-mTOR in AI cells only (Figure 4). *p*-XSC also causes decreased amounts of mTOR complex 2 protein Rictor to be co-immunoprecipitated with mTOR in AR and AI cells (Figure 5). Again, SM showed the same effect in AI cells only. Additionally, *p*-XSC mediated inhibition of mTOR phosphorylation is attenuated by androgen stimulation (10 nM dihydrotestosterone for 1 hr) in AR but not AI cells, supporting a link between the two signaling pathways in these cells.

Specific Aim 1c: Investigate the effect of SM and p-XSC on androgen receptor activity

We initially proposed to ascertain the effects of SM and *p*-XSC on androgen receptor transcriptional activity using a luciferase reporter assay. Transfection of the LNCaP and LNCaP C4-2 cell lines with the ARE-luciferase reporter plasmid has proved immensely difficult and the conditions for this assay have yet to be standardized in our lab. While working on resolving this issue we have begun to examine the effects of our

compounds on androgen receptor transcriptional activity by measuring the expression of prostate specific antigen (PSA), an androgen receptor target gene. Preliminary results show that SM and *p*-XSC inhibit PSA expression in AR (Figure 6A) and AI cells (Figure 6C). It appears that this inhibition is enhanced by stimulation with androgen in AR cells (Figure 6B) but not AI cells (Figure 6D).

Specific Aim 1d: Study the effects of SM and p-XSC on the DNA binding activity of transcription factors involved in the regulation of androgen receptor expression

Summary*: p-XSC inhibits recombinant Sp1 activity more effectively than SM; both compounds had no effect on endogenous Sp1 activity.*

Details: Several transcription factors are known to bind to elements of the androgen receptor gene/promoter (e.g., Sp1, Sp3, NFκB, and neurofibromatosis 1), however Sp1 appears to be the major regulator of androgen receptor transcription (28-30). We analyzed the effects of *p*-XSC on the DNA binding activity of recombinant Sp1 protein and Sp1 from nuclear extracts of cells treated with the compound using an ELISA-based transcriptional activity assay. *p*-XSC significantly and more effectively than SM or methylseleninic acid inhibited the activity of recombinant Sp1 activity at 2.5 and 5 µM concentrations (Figure 7A). However, at the same doses, it showed no inhibition of endogenous Sp1 activity in both AR (Figure 7B) and AI cells (Figure 7C).

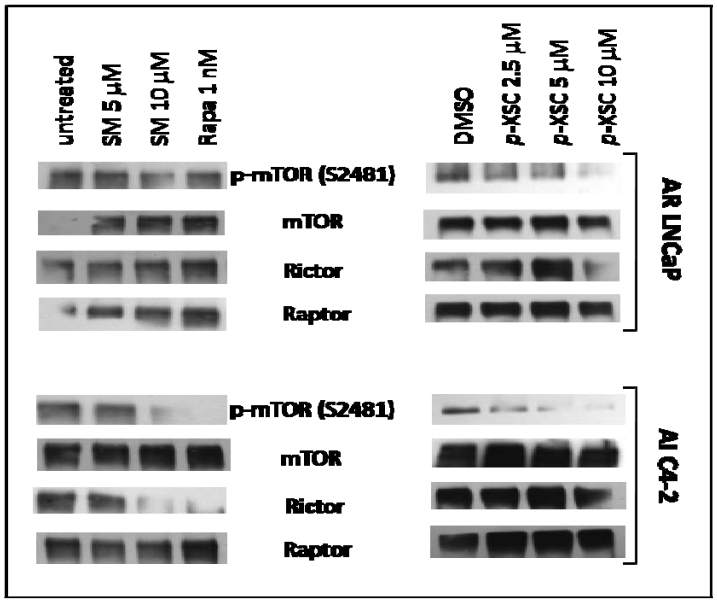


Figure 5. Immunoprecipitated mTOR protein from selenium or rapamycin (rapa) treated AR and AI cell lysates was analyzed, by western blotting, for phosphorylation and binding to mTOR complex proteins Raptor and Rictor.

DISCUSSION

Our results up to this point show that SM and *p*-XSC inhibit, potentially by different mechanisms, the growth of both AR and AI human prostate cancer cells. The effects of these agents on androgen receptor regulation differ depending on both the structure of the selenium compound and the androgen status of the cells line. Data shows that in AR cells both compounds decrease androgen receptor protein levels and PSA expression, which primarily regulated by the androgen receptor. The inhibition of PSA gene transcription is further enhanced in AR cells stimulated by androgens, suggesting that the selenium-mediated down-regulation of expression occurs at least in part through the androgen

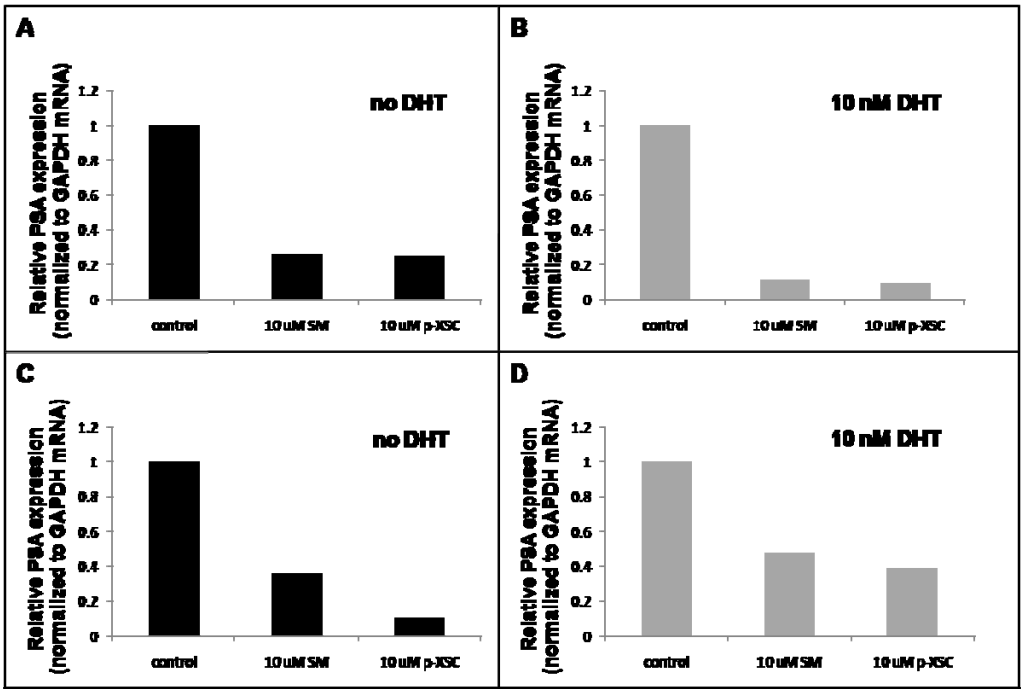


Figure 6. PSA mRNA levels measured by quantitative real time PCR in AR cells without (A) or with (B) stimulation with dihydrotestorserone (DHT) and in AR cells without (C) or with (D) stimulation with DHT.

receptor signaling pathway. Though preliminary data show that SM and *p*-XSC also decrease PSA expression in AI cells, only *p*-XSC is able to downregulate androgen receptor protein. Additionally, inhibition of PSA expression in AI cells does not appear to be affected by stimulation with androgens. Although there is currently a poor understanding of what drives the transition to androgen independence in prostate cancer, androgen bypass through altered activity of and crosstalk with other signaling pathways (e.g., PI3K/Akt, MAPK) has been suggested to play a role (14). Therefore, it is possible that selenium-mediated inhibition of androgen receptor transcriptional activity may occur through modulation of one or more of these pathways.

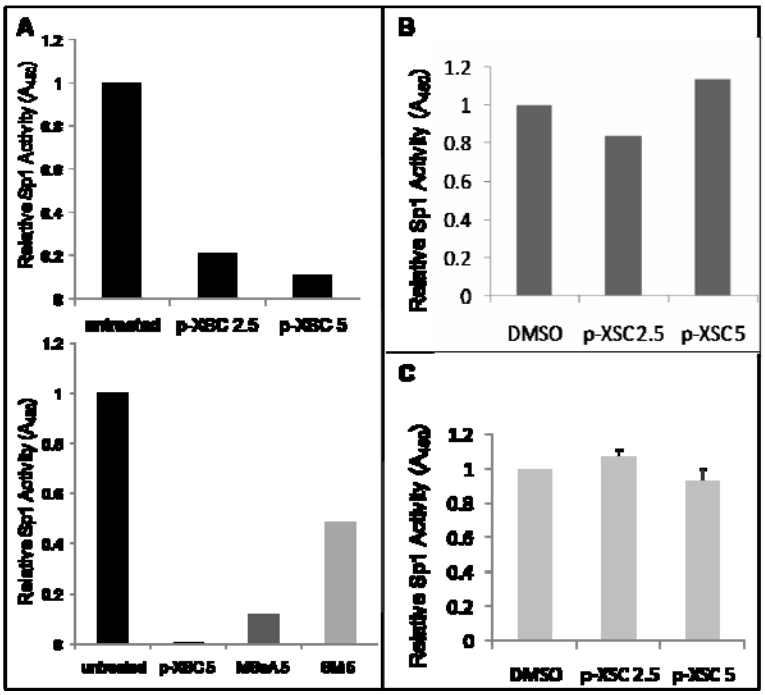


Figure 7. Activity of A. recombinant Sp1 protein incubated with selenium compounds and endogenous Sp1 protein in nuclear extracts from B. AR and C. AI cells treated with *p*-XSC.

Investigation of the effects of SM and *p*-XSC on Akt signaling showed no inhibition by either compound of Akt expression, phosphorylation, or kinase activity in AR and AI cells. It is possible that we were unable to see inhibition of Akt activation at the same time points at which these compounds elicit their down-stream effect on proliferation and apoptosis because it may be a very early obstructive event. We believe it will be informative to examine the effects of SM and *p*-XSC at earlier time points and plan to do so in future experiments. We have yet to see evidence that SM and/or *p*-XSC act though an Akt-dependent mechanism, however, our data shows clear effects of these compounds on proteins involved in the mTOR signaling pathway, another pathway known to have considerable crosstalk with androgen receptor signaling in the prostate. As with androgen receptor regulation, the effects of SM and *p*-XSC on mTOR signaling appear to depend on the structure and dose of the compound and the androgen status of the cell line. Additionally, these compounds may be targeting specific arms of the mTOR pathway (i.e., mTOR complex 1 vs. mTOR complex 2). Both compounds have the ability to inhibit mTOR phosphorylation in AR and AI cells but AI cells appear slightly more sensitive. *p*-XSC decreases the levels of the protein Rictor complexed with mTOR in AR and AI cells, whereas only SM inhibits the binding of the Rictor in AI cells. Inhibition of mTOR complex 1 of which Rictor is a part can induce apoptosis in an Akt- and androgen receptor-dependent manner (27). Neither compound affected the levels of Raptor, an mTOR complex 2 protein, complexed with mTOR in AR and AI cells. These results suggest that selenium-mediated alterations in androgen signaling may be mediated by mTOR. Future studies will explore further the specific mechanisms of mTOR inhibition by selenium and discern whether this inhibition is linked to androgen receptor regulation in AR and AI prostate cancer cells.

KEY RESEARCH AND TRAINING ACCOMPLISHMENTS

Training

- Further acquired the skills to formulate hypotheses, perform independent experiments, and interpret data.

- Presented research findings and sought feedback at an international cancer research meeting (AACR 2009 Annual Meeting, Denver, CO) and in house during Department of Biochemistry and Molecular Biology's student seminar series and at the Annual Graduate Student Research Forum.

- Examined literature on the role of selenium on prostate cancer prevention and published a review article title "Potential stages for prostate cancer prevention by selenium: Implications for cancer survivors" in the peer-reviewed journal Cancer Research (Facompre N and El-Bayoumy K. Cacner Res. 2009;69:2699-703).

- Completed several of the tasks outlined in the SOW for this award in a timely fashion.

Research

- Determined that the apoptosis inducing capabilities of selenium compounds is dependent on dose and form.

- Demonstrated that hat the selenium compounds SM and *p*-XSC can down-regulate androgen receptor protein levels, phoshporylation and PSA gene expression in human prostate cancer cells and that these effects are dependent on both the structure of the selenium compound and the androgen status of the cell line.

- Showed that SM and *p*-XSC do not alter Akt activity at the doses and time points examined.

- Resolved that though *p*-XSC can inhibit recombinant Sp1 activity, it does not down-regulate androgen receptor expression through inhibition of transcription factor Sp1 in cell culture.

- Showed, for the first time, that selenium compounds can inhibit mammalian target of rapamycin (mTOR) phosphorylation and complex formation in human prostate cancer cells.

REPORTABLE OUTCOMES

Poster Presentations

- **Facompre N,** Sinha I, El-Bayoumy K, and Sinha R. *Selenium targets mTOR signaling in prostate cancer cells.* 100th Annual Meeting of the American Association for Cancer Research, Dencer, CO, April 18-22, 2009

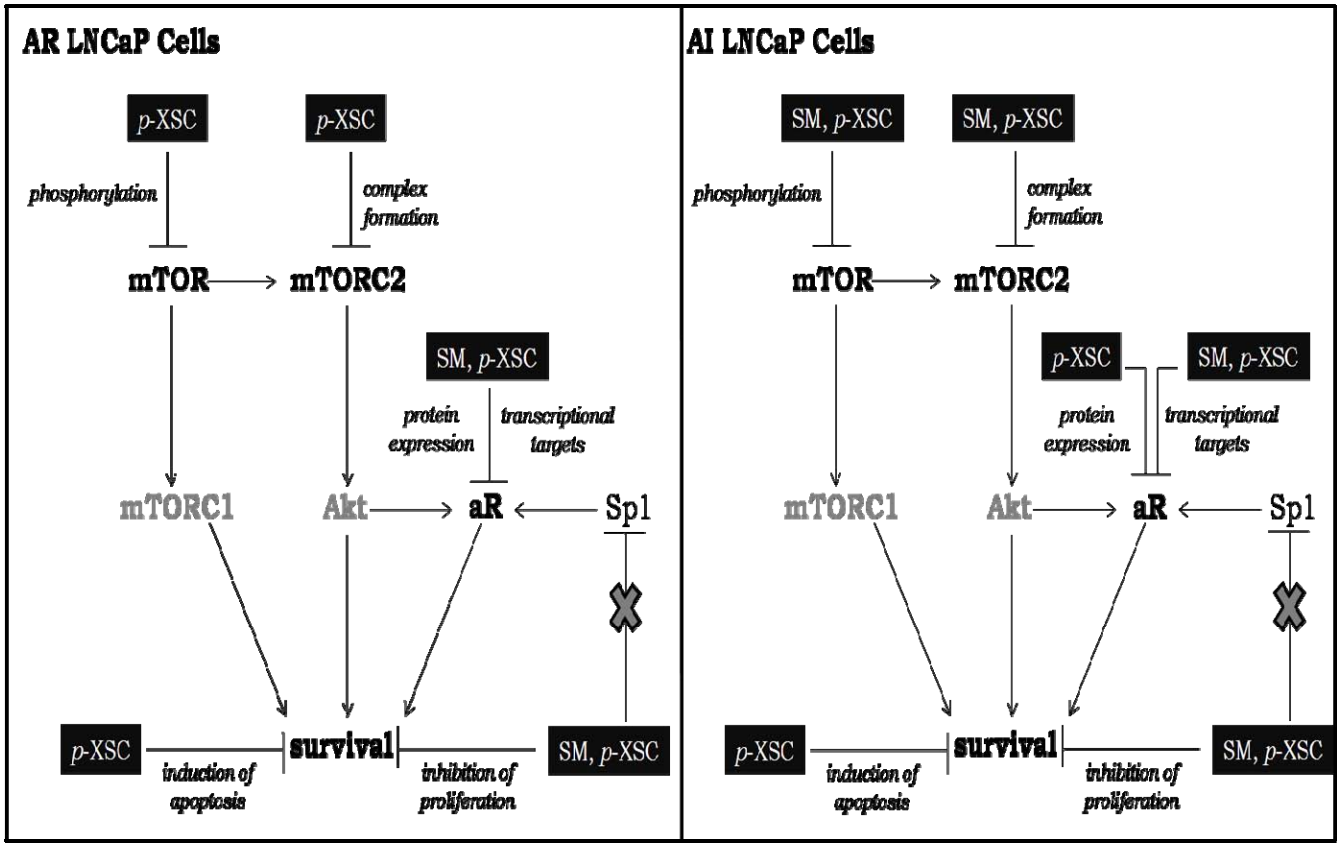
- **Facompre N**, Sinha R, and El-Bayoumy K. *Differential effects of naturally occurring and synthetic organoselenium compounds on androgen receptor regulation in prostate cancer cells.* 21st Annual Graduate Student Research Forum, Hershey, PA, March 6, 2009

Oral Presentations

- **Facompre N**. *Differential effects of naturally occurring and synthetic organoselenium compounds on androgen receptor in prostate cancer cells.* Department of Biochemistry and Molecular Biology Student Seminar Series, Penn State College of Medicine, Hershey, PA, July 7, 2008

CONCLUSION

The findings of the current study support the established concept that dose and form are critical for the anti-cancer effects of selenium compounds. The organoselenium compounds SM and *p*-XSC differentially interfere with prostate cancer cell growth and androgen receptor signaling. We have shown, for the first time, that inhibition of androgen receptor signaling by these compounds may be due, in part, to modulation of the mammalian target of rapamycin (mTOR) pathway. Our findings support the notion that selenium compounds may be of value, either individually or in combination with other therapies, for the treatment of prostate cancer because of their potential to inhibit critical prostate signaling pathways (Scheme 1). Detailed mechanistic studies comparing different selenium compounds may provide a better understanding of clinical outcomes, particularly a possible rationale for the ineffectiveness of SM (alone and in combination with vitamin E) as a prostate chemopreventive agent in the recently halted Selenium and Vitamin E Cancer Prevention Trial (SELECT) (31). The SELECT study was initiated after secondary results of the Nutritional Prevention of Cancer study reported selenium-enriched yeast, which contains various forms of selenium, reduced prostate cancer risk by 63% (32). Future studies will continue to uncover the role of cell signaling pathways known to exhibit crosstalk with androgen receptor signaling (e.g., Akt and mTOR) in selenium-mediated inhibition of prostate cancer cells survival. Ascertaining the specific molecular targets of structurally distinct organoselenium compounds as well as selenium-enriched yeast will be critical for the validation of selenium as a preventative or therapeutic modality for prostate cancer and for the development of more efficacious agents.



Scheme 1. Representation of the differential mechanistic effects of SM and *p*-XSC on survival pathways in AR and AI human prostate cancer cells accounting for growth inhibition and induction of apoptosis. Targets in **Bold** are altered by selenium compounds; targets in **Grey** have not yet been tested or require further investigation.

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APPENDIX A: Abstracts

2009 Annual Graduate Student Research Forum
March 6, 2009
Hershey, PA

Presentation Title: Differential effects of naturally occurring and synthetic organoselenium compounds on androgen receptor regulation in prostate cancer cells

Presentation Start/End Time: Friday, Mar 6, 2009, 3:00 PM - 5:00 PM

Author Block: *Nicole Facompre*, *Indu Sinha*, *Karam El-Bayoumy*, *Raghu Sinha*. Pennsylvania State University College of Medicine, Hershey, PA

In the United States, prostate cancer is the most commonly diagnosed malignancy and second leading cause of cancer related death in men. Chemoprevention is a plausible approach to block or delay the process of cancer development. Epidemiologic analysis, preclinical studies, and some clinical intervention trials show a protective role for selenium against prostate cancer. However, the mechanisms that account for cancer prevention by selenium remain unclear. The objective of the present study is to elucidate the mechanisms by which structurally distinct naturally occurring and synthetic organoselenium compounds exert their anti-proliferative and/or pro-apoptotic effects on androgen responsive (AR) and androgen independent (AI) prostate cancer cells. Toward this goal, using AR LNCaP and AI LNCaP C4-2 human prostate cancer cells, we examined the effects of a range of doses of selenomethionine (SM), a naturally occurring form of selenium, and 1,4-phenylenebis(methylene)selenocyanate (*p*-XSC), a synthetic compound and superior agent to SM in numerous preclinical animal models on cellular and molecular targets that are critical in the development of prostate cancer. We have shown that both SM and *p*-XSC inhibit AR and AI cell proliferation, though *p*-XSC is effective at significantly lower does. We further demonstrated that these compounds have differential effects on apoptosis and cell cycle distribution. Also, SM and *p*-XSC appear to inhibit androgen receptor signaling through different mechanisms. *p*-XSC down-regulates androgen receptor protein expression in both AR and AI prostate cancer cells while SM decreases androgen receptor levels in AR cells only. SM and *p*-XSC also inhibit Akt-mediated phosphorylation of androgen receptor, which may play a role in modulating its regulation. However, only SM caused a decrease in phosho-Akt levels. Together these results indicate that the direct effects of selenium compounds on prostate cancer cells differ depending on their structure and the androgen status of the cell line. We are further exploring the effects of these compounds on pathways that regulate the androgen receptor (e.g., mTOR signaling, Sp1 and NFκB-mediated transcription) and that may be targeted by selenium. Preliminary data shows that SM and *p*-XSC can inhibit mTOR and its downstream target p70S6 kinase in AR and AI cells. Also, SM and *p*-XSC directly inhibit the binding of recombinant Sp1 to DNA. Understanding the molecular mechanisms of selenium-mediated down-regulation of androgen receptor signaling will provide further evidence for its potential role against prostate cancer either individually or in combination with other therapeutic regimens.

2009 AACR Annual Meeting
April 18-22, 2009
Denver, CO

Abstract Number:	5579
Session Title:	Novel Agents 4
Presentation Title:	The inhibitory effects of organoselenium compounds on mTOR signaling in prostate cancer cells
Presentation Start/End Time:	Wednesday, Apr 22, 2009, 8:00 AM -12:00 PM
Location:	Hall B-F, Poster Section 37
Poster Section:	37
Poster Board Number:	21
Author Block:	*Nicole Facompre*, *Indu Sinha*, *Karam El-Bayoumy*, *Raghu Sinha*. Pennsylvania State University College of Medicine, Hershey, PA

Prostate cancer is the most commonly diagnosed malignancy and the second leading cause of cancer-related death in men in the United States. Androgen receptor signaling is critical for prostate cancer cell growth and thus anti-androgen therapies are commonly used to treat localized disease. However, the cancer eventually relapses into a hormone-resistant state. Currently there is a lack of effective treatments for advanced and hormone-resistant prostate cancers. We have previously shown that selenomethionine (SM), and 1,4-phenylenebis(methylene)selenocyanate (p-XSC) down-regulate androgen receptor expression and its phosphorylation in androgen responsive (AR) and androgen independent (AI) cells. Preclinical studies and proteomic analyses of human prostate tissues have implicated the mammalian target of rapamycin (mTOR) signaling pathway in the progression of prostate cancer and its transition to androgen independence, suggesting mTOR as a potential target for new therapies. Experimental studies further demonstrate a cross-talk between mTOR and androgen signaling in prostate cancer cells. We hypothesize that selenium inhibits prostate cancer cell growth by interfering with the mTOR signaling pathway and this inhibition may be linked to down-regulation of androgen receptor signaling in these cells. Here we showed, for the first time, that selenium compounds (SM, p-XSC, and methylseleninic acid) inhibit phosphorylation of mTOR and its downstream target p70S6 kinase in both LNCaP (AR) and LNCaP C4-2 (AI) human prostate cancer cells. Selenium-mediated inhibition of mTOR is attenuated by stimulation with androgens in AR but not in AI cells. Experiments are underway to determine if there is a link between mTOR inhibition by selenium and selenium-mediated modulation of androgen receptor signaling. In addition, the effects of different forms and doses of selenium on both mTOR complexes (mTORC1 and mTORC2) and their components are being examined. Validation of mTOR as a target of selenium in prostate cancer will provide evidence for its potential role against advanced and hormone refractory prostate disease either individually or in combination with anti-androgens.
Support: DOD PC074101, NCI R01127729, Penn State Hershey Cancer Institute Funds

APPENDIX B: Curriculum Vitae

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Graduate Student in Biochemistry and Molecular Biology
Department of Biochemistry and Molecular Biology, H171
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Education

Ph.D. candidate, Biochemistry and Molecular Biology — 2004 - Present
Hershey Medical Center, Pennsylvania State University (PSU) — **GPA:** 3.66

B.S., Biochemistry — 2000-2004
Minor, Science Writing — **GPA:** 3.60
Lehigh University, Bethlehem, PA

Research Experience

2004-Present Graduate Assistant, PSU
2003 REU Research Fellow, Stony Brook University, Stony Brook, NY

Awards/Honors

2008-2011 Prostate Cancer Training Award, Department of Defense
2007 Graduate Research Supplement Award, PSU
2004 Graham Endowed Fellowship, PSU
2004-Present *Phi Beta Kappa* Academic Honor Society
2003 Henry Farmer Memorial Scholarship, Lehigh University
2001-2004 Lehigh University's Dean's List
2000-2004 Dean's Scholarship, Lehigh University

Leadership Experience

2008 Junior Mentor for the NIDDK Short-Term Education Program for Underrepresented Persons (STEP-UP), PSU
2006-2007 Assisted Dr. Raghu Sinha in mentorship of graduate rotation students
2005-2008 Historian, Graduate Student Association (GSA), PSU
2005-2008 GSA Representative to the College of Medicine Student Assembly, PSU
2005-2006 Department of Biochemistry Graduate Student Representative, PSU

Publications: Peer Reviewed Articles

Facompre N and El-Bayoumy K. Potential stages for prostate cancer prevention with selenium: implication for cancer survivors. *Cancer Res*. 2009; 69:2699-2703

Sinha R, Pinto JT, **Facompre N**, Kilheffer J, Baatz J, El-Bayoumy K. Effects of naturally occurring and synthetic organoselenium compounds on protein profiling in androgen responsive and androgen independent human prostate cancer cells. *Nutr Cancer* 2008; 60:267-275

Pinto JT, Sinha R, Papp K, **Facompre ND**, Desai D, El-Bayoumy K. Differential effects of naturally occurring and synthetic organoselenium compounds on biomarkers in androgen-responsive and androgen-independent human prostate carcinoma cells. *Int J Cancer.* 2007; 120:1410-1417.

Baessler KA, Lee Y, Roberts KS, **Facompre N**, Sampson NS. Multivalent Fertitlinβ Oligopeptides: The Dependence of Fertilization Inhibition on Length and Density. *Chem Biol* 2006;13:251-259.

Abstracts and Poster Presentations (Presenter underlined)

Facompre N, Sinha I, El-Bayoumy K, and Sinha R. *Selenium targets mTOR signaling in prostate cancer cells.* 100th Annual Meeting of the American Association for Cancer Research, Dencer, CO, April 18-22, 2009

Sinha R, Sinha I, **Facompre N**, Russell S, Somiari R, King T, Richie J, and El-Bayoumy K. *Selenium-enriched yeast inhibits α-1 antitrypsin to a varied extent in healthy African Americans and Caucasians.* 100th Annual Meeting of the American Association for Cancer Research, Dencer, CO, April 18-22, 2009

Facompre N, Sinha R, and El-Bayoumy K. *Differential effects of naturally occurring and synthetic organoselenium compounds on androgen receptor regulation in prostate cancer cells.* 21st Annual Graduate Student Research Forum, Hershey, PA, March 6, 2009

El-Bayoumy K, Richie J, Sinha R, **Facompre N**, Chen K, Somiari, Reese C and Muscat J. *Mechanisms of Prostate Cancer Prevention by Selenium: Implications for Cancer Survivorship*. AICR 2008 Research Conference on Food, Nutrition, Physical Activity & Cancer, Washington DC, November 6-7, 2008

Facompre N, Sinha R, Null, K, Pinto J, and El-Bayoumy K. *Differential effects of naturally occurring and synthetic organoselenium compounds on androgen receptor in prostate cancer cells*. 99th Annual Meeting of the American Association for Cancer Research, Sand Diego, CA, April 12-16, 2008.

Facompre N, Sinha R, Null, K, Pinto J, and El-Bayoumy K. *Differential effects of naturally occurring and synthetic organoselenium compounds on androgen receptor in prostate cancer cells*. 20th Annual Graduate Student Research Forum, Hershey, PA, March 7, 2008.

Facompre N, Null K, Pinto J, Sinha R, and El-Bayoumy K. *Differential effects of naturally occurring and synthetic organoselenium compounds on androgen receptor in prostate cancer cells*. 4th Annual Penn State Cancer Institute Retreat, Hershey, PA, November 28-29, 2007.

Sinha R, **Facompre N**, Kilheffer J, Baatz, J, Russell S, Somiari R, Richie J and El-Bayoumy K. *Selenized-yeast supplementation alters serum pre-serum amyloid P component and alpha-1-antitrypsin in healthy individual*. 98th Annual Meeting of American Association for Cancer Research, Los Angeles, CA, April 14-18, 2007.

Facompre N, Sinha R, Desai D, Amin S, and El-Bayoumy K. *Differential effects of naturally occurring and synthetic organoselenium compounds on androgen receptor in prostate cancer cell*s. 19th Annual Graduate Student Research Forum, Pennsylvania State University College of Medicine, Hershey, PA, March 2, 2007.

Sinha R, **Facompre N**, Kilheffer J, Baatz J, Russell S, Somiari R, Richie J, El-Bayoumy K. *Serum Proteomic Profile of Selenized-Yeast Supplemented Healthy Men.* Crossover 2006, State College, PA, October 12, 2006.

Sinha R, Smith JS, **Facompre N**, El-Bayoumy K, Null K, Grenko R, Kass R, King T, Keshava C. *Investigating selenium compounds against development of early breast cancer in a three dimensional collagen gel model*. 7th International Selenium Symposium, Madison, WI, October 25-30, 2006.

Sinha R, **Facompre ND**, King T, Ganther H, El-Bayoumy K. *Mechanism-based growth inhibition of premalignant human breast disease by organoselenium compounds*. 97th Annual Meeting of American Association for Cancer Research, Washington, D.C. April 1-5, 2006.

Sinha R, Smith JS, **Facompre ND**, Grenko R, Kass R, King T, El-Bayoumy K. *A three-dimensional collagen gel model for investigating potential chemopreventive agents against development of early breast cancer*. 97th Annual Meeting of American Association for Cancer Research, Washington, D.C. April 1-5, 2006.

Facompre N, Sinha R, El-Bayoumy K. *1,4-phenylenebis(methylene)selenocyanate and docosahexaenoic acid: A potential combination strategy for the prevention of breast cancer.* 18th Annual Graduate Student Research Forum, Pennsylvania State University College of Medicine, Hershey, PA, March 3, 2006.